FACTORS OF EQUATORIAL CONTRACTION AND POLAR MEMBRANE EXPANSION IN ENDOSPERM CYTOKINESIS

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In a recent review (Roberts, 1961) I suggested that cytokinesis in both plant and animal cells might be explained in terms of the factors of polar expansion, equatorial contraction, and new membrane formation. Mazia (1961) has also discussed basic similarities in cytokinesis of plant and animal cells. There is considerable support for the idea that new membrane formation may occur in the connecting stalk of dividing animal cells late in cleavage (references and discussion in Roberts, 1961; Mazia, 1961; Buck and Tisdale, 1962a, 1962b). Buck and Tisdale (1962a) have demonstrated in rat erythroblasts the formation of a midbody in the equator of the spindle during cleavage, which appears to correspond in structure to the phragmoplast of plant cells, and (1962b) have described in three types of mammalian cells membrane-bound vesicles, apparently derived from endoplasmic reticulum, which develop in the cleavage plane and apparently are the source of new cell membrane in the furrow region. Their observations are somewhat similar to those of Porter and Machado (1960) on cell-plate formation in *Allium*. However, there has been almost no evidence that polar expansion and equatorial contraction might play a part in cytokinesis of plant cells which employ cell-plate formation, other than the observations of Bajer and Molè-Bajer (1956) on the division of well flattened living endosperm cells. *"The lack of cellulose walls makes possible the formation of cytoplasmic protuberances. These pseudopodia-like strings of cytoplasm may form during the whole division, but are most prominent in prophase and telophase. During pro- and metaphase the shape of the cell often changes to a sphere or an ellipsoid—often without pseudopodia formation. It is of interest that in other material the cells often become round before metaphase. In animal tissue it is well known that pseudopodia disappear before anaphase. . . . Endosperm cells often form outpushings during the telophase."* These suggestions of surface forces in dividing endosperm, similar to those of dividing animal cells, stimulated the writer to look at dividing living endosperm cells which had not been distorted by flattening.

Investigators are rightfully concerned with standards of normalcy in studies of living cells, and with conclusions based on cells studied under abnormal conditions. Some would reject all studies of cell division based on cells which do not complete at least two divisions without evidence of abnormality. Such a criterion would impose serious and frequently impossible difficulties on the study of terminal divisions, as in secondary spermatocytes, and eliminate as subjects of investigation many cells with long division cycles. Other investigators recognize that degrees of abnormality are the almost inevitable result of preparation and observation. They believe that there is information to be gained from abnormal cells, provided

the investigator recognizes and makes extremely clear the limits of his methods and of his results, and is extremely cautious in his conclusions. The observations here reported were made on cells which do not meet the criterion above and which were dividing under abnormal conditions. The limitations are detailed in the body of the paper and are considered in the discussion. Subject to these limitations the observations support the idea that equatorial contraction and polar expansion may play a significant role in cytokinesis of endosperm cells lacking a cell wall.

Materials and Methods

Young seeds still in the milk stage of whatever horticultural varieties of *Hemerocallis* were available at the moment, were the source of endosperm. Seeds collected at mid-day or early afternoon provided the best material. Those collected in the early morning contained few dividing cells. One end of the seed was sliced off with a sharp scalpel, and endosperm was sucked out of the cavity with a 1-ml. syringe fitted with a 24-gauge needle whose tip had been ground to roundness. The seeds were not squeezed to express more material. Four to eight seeds provided enough material for a preparation. A 5–7-mm. square was outlined on a no. 1 coverslip with Vaseline extruded through a 24-gauge hypodermic needle, and the enclosed space flooded with endosperm fluid. Excess fluid was carefully withdrawn with the syringe, leaving a relatively flat film whose depth could be adjusted with some experience. The coverslip was then inverted over the shallow depression of a Fisher-Littman well slide and sealed by a ring of Vaseline. Preparations were examined and photographed by phase contrast microscopy using a 12.5 × compensating ocular and 45 × dark contrast objective. The microscope (Spencer) was equipped with long focal length phase plates. Illumination was provided by a ribbon filament lamp with 36 mm. of 3% copper sulfate solution in the light path as a heat absorber. Light intensity was controlled by a variable transformer and was kept at the minimum possible for observation except for the few seconds required for photographic exposure.

The preparations differ significantly from those of the Bajers (1954; 1956). Thick hanging drop preparations were necessary to avoid flattening. This introduced problems already described by Bajer (1954, p. 386). ". . . *the thickness of the drops is of greatest importance. In large drops the penetration of oxygen is not sufficient, and in consequence most cells die in prophase, though most mitoses in metakinesis are continued to telophase.*" Bajer found it necessary to use drops only six micra in thickness. Since freely suspended cells were essential a soft agar substrate could not be used. Most of the first season of study was devoted to efforts to achieve longer-lived preparations, without significant success. It was thus necessary to accept the limitations imposed by the requirement for thick preparations and freely suspended cells, and resort to the simple preparations described. Although the small air space of the thin slides used was rapidly saturated, as judged by condensation, evaporation ensured that the medium was at least mildly hypertonic. Preparations thus made were short-lived. After 1½–2 hours the cells showed obvious signs of abnormality and division soon ceased. Cells first observed in prophase could be followed to metaphase. Those first observed in metaphase could be followed to late anaphase. Cells in middle or late anaphase could be followed to telophase and completion of the cell plate.

Routinely each preparation was completely scanned by overlapping traverses and the location of suitable cells for further observation and photography noted. Such scanning was completed in approximately five minutes. It is emphasized that all of the stages described were regularly observed during the preliminary scanning, long before there were evidences of abnormality. The investigation extended through two summers. Seventy cells were followed in detail, and 13 divisions recorded photographically. Incidental observations and photographs were made of many more. Only those cells which continued to divide, subject to the limitations previously described, are the basis of the following observations.

Observations and Discussion

The preparations contained abundant particulate material, ranging in size from barely visible to 1–2 micra, cellular debris, bits of peripheral endosperm tissue with well developed cell walls, and free nuclei. The numerous small particles are a source of difficulty in observing and photographing cells deep in the fluid but

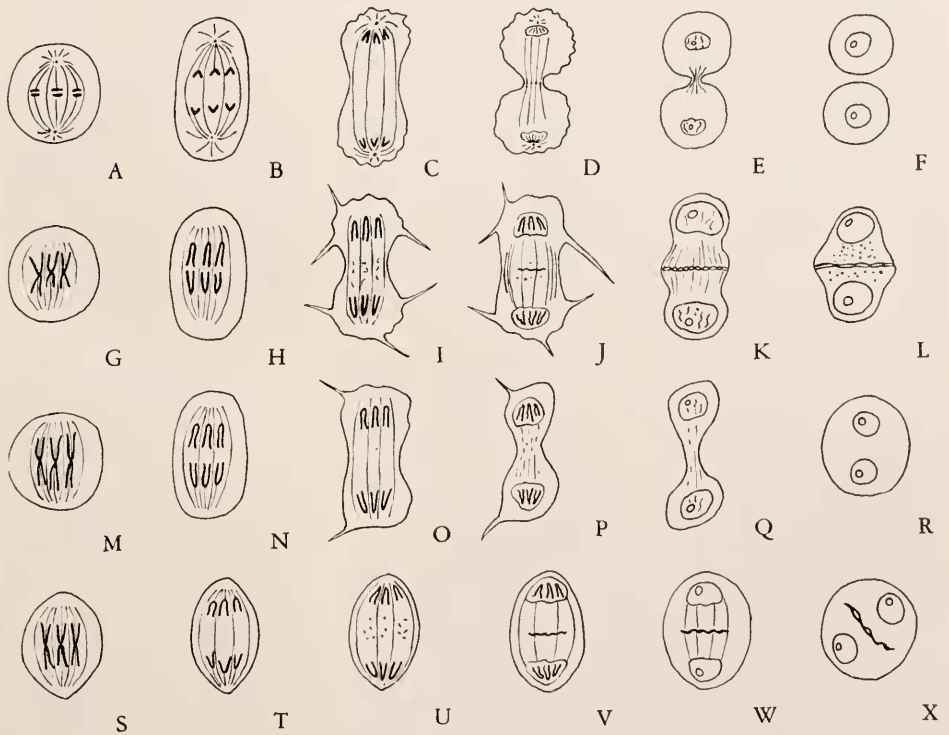
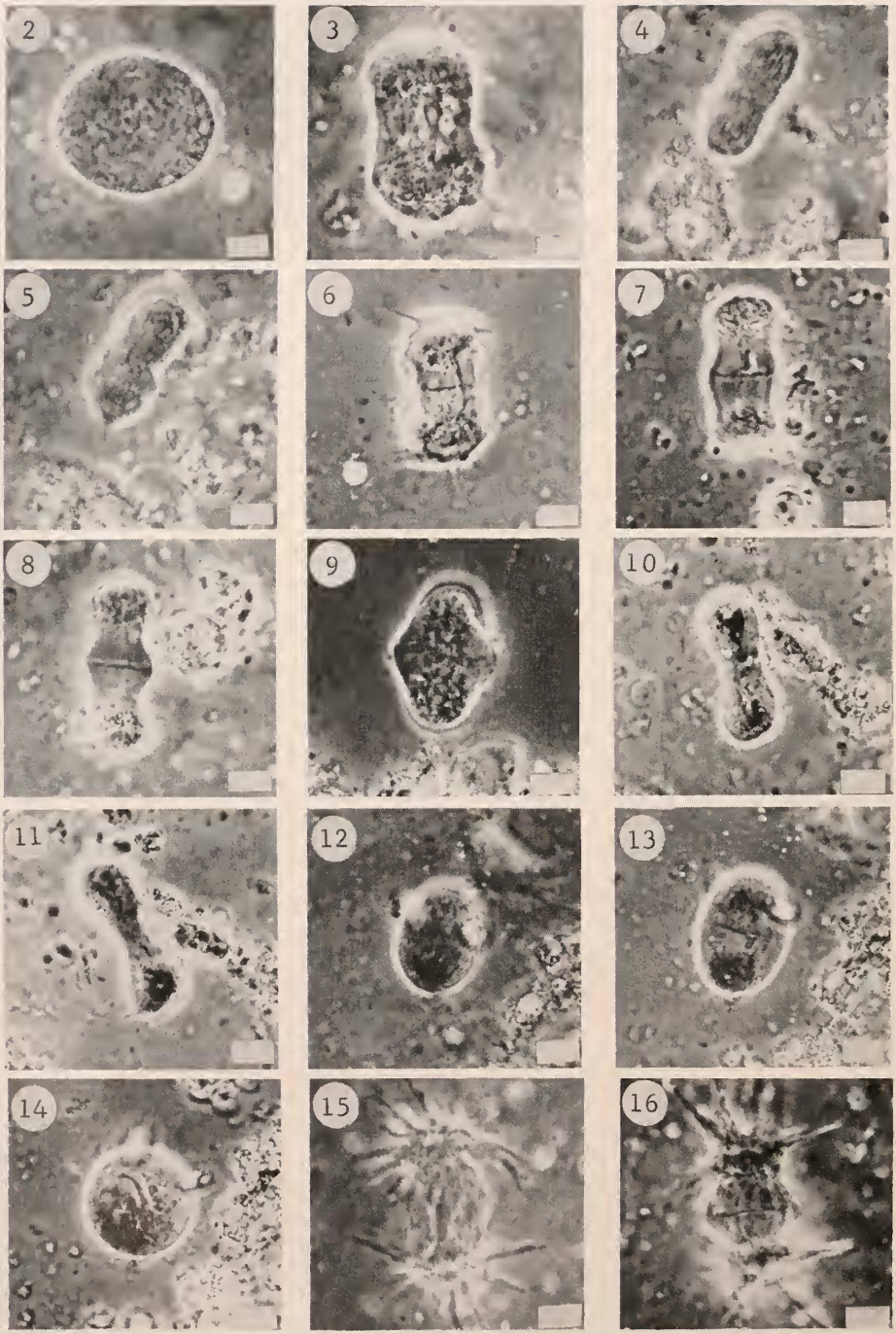


Figure 1. Comparison of cytokinesis of animal cells and endosperm. A-F, cytokinesis of animal cells. In C-D polar membrane expansions, which may take different forms or may be demonstrable only indirectly in various cell types, are shown as surface irregularities. G-L, cytokinesis in endosperm. Equatorial constriction and the two types of surface irregularities are shown in I-J. M-R, failure of cell plate formation and of cytokinesis in endosperm, based on a single cell. S-X, absence of equatorial constriction and polar irregularities followed by failure of cytokinesis.



FIGURES 2–16.

the presence of the smallest particles in active Brownian movement above the cells being observed ensured that they were not compressed between the coverslip and the surface film. The free nuclei were not observed to divide nor were recognizable prophases of division seen. It is worthy of comment that most of these nuclei had adherent granular material which could not be optically distinguished from the smaller particles seen in the endosperm fluid but lacked visible cell membranes. Division was observed only in *cells* with cytoplasm and enclosed in a cell membrane. The cells, when flattened, were entirely comparable to those studied by the Bajers (1954, 1956) and are believed to be of the same type. Their origin within the endosperm sac is unknown but is presumed to be the same gelatinous layer which the Bajers expressed from the seed.

Figure 1 summarizes the observations on the process of cytokinesis and provides a comparison with similar stages in animal cells. At metaphase the cells round up (Figs. 1G, 2), much as described in the previous quotation from Bajer. During anaphase the cells elongate (Fig. 1H), and in late anaphase or early telophase develop a distinct equatorial constriction (Figs. 1, I–J, 3–5), entirely comparable in appearance to early furrow formation in animal cells. At about the same time irregularities appear in the membrane at the cell poles. These irregularities are of two types; small bulges which appear in the same location and at the same stage of division as the blebs and visible expansions characteristic of cleaving animal cells, and long oriented spinous processes (Figs. 1 I–J, 3–6). Small particles, which have become increasingly visible in the spindle, aggregate in the equator and fuse to form the cell-plate which extends peripherally, eliminating the equatorial constriction and producing a pronounced bulge at the equator (Figs. 1K, 7–8). The polar membrane irregularities gradually disappear, leaving a configuration like that seen in Figures 1K, 7–8. This gradually shortens to the condition seen in Figures 1L, 9, the final stage which could be followed. Except that they are at all times enclosed in a cell membrane, the configurations assumed by the dividing cells are quite comparable to those shown by Jungers (1931).

The similarity to animal cell cytokinesis is striking and is well illustrated in Figure 1. The manner in which such an illustration is drawn can dramatize similarities and de-emphasize differences, but reference to the photographs (Figs. 2–16) should be convincing, especially to those who have observed division of freely suspended animal cells. The observations establish the fact of equatorial

FIGURES 2–3. Metaphase and anaphase of the same cell. Figure 3 shows equatorial constriction, membrane irregularities and one spinous process. In these and the following figures the scale, 480 ×, is shown by the small rectangles, whose length indicates 10 μ.

FIGURES 4–5. Middle and late anaphase of the same cell, showing development of equatorial constriction and polar irregularities. Comparison with Figures 2–3 indicates range of cell size observed.

FIGURE 6. Cell plate formation in early telophase. The spinous processes are well developed but not unusually so.

FIGURES 7–9. Telophase and the completion of cytokinesis. Figure 9 is the same cell shown in Figure 6.

FIGURES 10–11. Failure of cell plate formation and maximum observed equatorial constriction. In this cell cytokinesis failed and a binucleate resulted.

FIGURES 12–14. Absence of equatorial constriction and membrane irregularities. The cell plate in Figure 14 appeared to be degenerating.

FIGURES 15–16. "Amphiastral" configurations in medium made hypertonic with glucose. The spinous processes suggest astral rays.

constriction but of themselves tell us nothing of its nature. It does not appear to result from conformation of the cell membrane to the contours of the internal spindle and re-forming nuclei. Small particles in Brownian movement may occasionally be seen between the spindle and equatorial constriction, and around the re-forming nuclei. There is evidence the equatorial constriction in animal cells is the result of an equatorial contraction (reviewed in Roberts, 1961). I suggest that the same mechanism may be operative in these endosperm cells, probably to a lesser degree. The observed changes in the polar cell membranes undoubtedly are accompanied by an increase in membrane area at the poles. Their occurrence at the same stage of division as the better known polar expansions of animal cells suggests they may play a similar but perhaps less important role in cytokinesis of endosperm cells. The long oriented spinous polar processes deserve special comment. They are straight, or nearly so, and if the long axes are projected backward, all converge in the re-forming nucleus. Occasionally in routine preparations and more commonly in preparations made more hypertonic by the addition of glucose (Figs. 15–16), the spines develop so abundantly that the cells resemble diagrammatic amphiastral figures. Wilson (1900) described somewhat similar "filose" processes extending from the poles of the elongated polar bodies and early blastomeres of *Cerebratulus*. The centers of "filose" activity were correlated with the position of the spindle poles of the amphiastral spindles. Lima-de-Faria (1958), Östergren (1954), and Östergren, Koopmans and Reitalu (1953) have described astral rays in numerous genera and species of higher plants. They are in agreement that the astral rays are small at metaphase and reach maximum development at late anaphase. Appearance and time of development suggest that the observed spinous processes of endosperm cells may be associated with astral development. However, astral rays have not been observed by the writer and other explanations are possible. Occasionally cells have been observed to divide with only minor polar membrane irregularities, without the development of spinous processes.

In a single instance a cell (Figs. 1 M–R, 10–11) was observed to progress to late anaphase quite normally and then fail to form a cell plate. During early telophase the constriction slowly deepened, advancing further than usual but falling far short of dividing the cell into two (Fig. 11). During telophase the cell suddenly rounded up, resulting in a binucleate. More frequently cells, identifiable in early anaphase by their distinctive outlines (Figs. 1 S–X, 12–14), failed to develop either irregularities of the polar membrane or equatorial constrictions. A cell plate formed quite normally but in all cases failed to contact the equatorial cell membranes. In telophase the cells rounded up and the cell plate showed signs of degeneration. Presumably they too would have formed binucleates, but the life of the preparations was too short to observe the final result. Occasionally binucleate cells were observed during the first minutes of a fresh preparation. It may be coincidental but is probably of significance that in every case in which one of the factors of equatorial constriction, polar membrane expansion, or cell plate formation failed, cytokinesis failed.

Observations such as those reported cannot of themselves establish that equatorial contraction and polar membrane expansions occur and are factors in the mechanism of cytokinesis of normal endosperm cells. The limitations of the methods used are such that the observations reported could be ascribed to the effects

of the methods employed. However, it is not without significance that in hundreds of preparations, during two seasons, every cell observed to divide did so as described. All of the stages described have been observed during the first few minutes after preparation. Further, the observations reported were made during the $1\frac{1}{2}$–2 hours before signs of deterioration appeared. On this basis the observations do suggest a strong probability that equatorial contraction and polar membrane expansions occur normally and are significant factors in the mechanism of cytokinesis of endosperm cells.

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Summary

Divisions of living endosperm cells of *Hemerocallis* have been observed in hanging drop preparations where they are not distorted by flattening. Equatorial constriction and polar membrane expansions occur in late anaphase and appear quite comparable to corresponding stages of cytokinesis in animal cells. Failure of equatorial constriction, of polar membrane expansion, or of cell plate formation results in failure of cytokinesis. It is suggested that equatorial contraction and polar membrane expansion may be functionally important in the mechanism of cytokinesis of endosperm cells, as they are in animal cell cytokinesis.

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